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Consumption and Metabolism of 1,2-Dimethoxy-4-(3-Fluoro-2-Propenyl)Benzene, a Fluorine Analog of Methyl Eugenol, in the Oriental Fruit Fly *Bactrocera dorsalis* (Hendel)

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Abstract Methyl eugenol (ME) is a natural phenylpropanoid highly attractive to oriental fruit fly *Bactrocera dorsalis* (Hendel) males. The flies eagerly feed on ME and produce hydroxylated metabolites with both pheromonal and allomonal functions. Side-chain metabolic activation of ME has long been recognized as a primary reason for hepatocarcinogenicity of this compound on rodents. In an attempt to develop a safer alternative to ME for fruit fly management, we developed a fluorine analog 1,2-dimethoxy-4-(3-fluoro-2-propenyl)benzene (I), which, in earlier field tests, was as active to the oriental fruit fly as ME. Now we report that *B. dorsalis* males are not only attracted to, but also eagerly consume (up to ~1 mg/insect) compound I, thus recognizing this fluorinated benzene as a close kin of the natural ME. The flies metabolized the fluorine analog I in a similar fashion producing mostly two hydroxylated products, 2-(3-fluoro-2-propenyl)-4,5-dimethoxyphenol (II) and (*E*)-coniferyl alcohol (III), which they stored in rectal glands. However, the introduction of the fluorine atom at the terminal carbon atom of the double bond favors the ring hydroxylation over a side-chain metabolic oxidation pathway, by which coniferyl alcohol is produced. It also appears that fluorination overall impedes the metabolism: at high feed rate (10 μl per 10 males), the flies consumed in total more fluorine analog I than ME but were unable to metabolize it as efficiently as ME.

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Introduction

The oriental fruit fly *Bactrocera dorsalis* (Hendel) is a wide-spread pest of a broad range of tropical, subtropical, and temperate host plants (US Department of Agriculture, 1983; White and Elson-Harris, 1992). Males of *B. dorsalis* are strongly attracted to and compulsively fed on methyl eugenol (ME) (Steiner, 1952), a commonly occurring plant phenylpropanoid (Metcalf, 1990). ME is also a common component of spices and is being used as food-flavoring agent at low concentrations (Hall and Oser, 1965). The use of ME in traps and killing stations assures successful detection, control, and eradication of the oriental fruit fly populations worldwide (Steiner et al., 1965; Koyama et al., 1984). The attraction to and consumption of ME by *B. dorsalis* and sibling species of the *dorsalis* complex, *B. papayae* and *B. carambolae*, have an ecological significance in the fly's communication system. It has been demonstrated that mature males after feeding on ME produced metabolites, mostly 2-(2-propenyl)-4,5-dimethoxyphenol (IV) and (*E*)-coniferyl alcohol (III), that display pheromonal and allomonal properties (Nishida et al., 1988a,b; Tan and Nishida, 1996). In addition, ME-acquired males have an earlier onset of courtship and are sexually more competitive than ME-deprived males (Tan and Nishida, 1996, 1998; Shelly, 2000).

Posing a potential problem to the continued use of ME in area-wide pest management programs are reports indicating that it causes hepatic tumors in mice (Miller et al., 1983), induces intrachromosomal recombination in a yeast assay (Schiestl et al., 1989), and elicits an abnormal response in a bacterial DNA repair test (Sekizawa and Shibamoto, 1982). The National Toxicology Program (NTP), US Department of Health and Human Services, evaluated ME and found that given orally to rats, it induced liver and stomach tumors in both sexes and kidney, mammary gland, and skin tumors in males (NTP, 1998). Recently, NTP listed ME as reasonably anticipated to become a human carcinogen based on sufficient evidence of its carcinogenicity in experimental animals (NTP, 2002). In 2001, the Expert Panel of the Flavor and Extract Manufacturers' Association performed a comprehensive review of all data relevant to safety evaluation of ME and concluded that daily intake of threshold doses that were carcinogenic in rodents was much higher than anticipated in human dietary intake. Hence, its potential to induce hepatotoxicity and carcinogenicity through dietary intake was expected to be negligible (Smith et al., 2002).

Since early warnings about the carcinogenicity of ME, a number of compounds have been evaluated as attractants for *B. dorsalis* (Mitchell et al., 1985; Khrimian et al., 1993, 1994; Liquido et al., 1998). In 1994, we discovered a fluorine analog, (*E*)-1,2-dimethoxy-4-(3-fluoro-2-propenyl)benzene (I-*E*), which in short field tests was as attractive as and more persistent than ME and, remarkably, was about twice as active as the *Z* isomer (Khrimian et al., 1994; Liquido et al., 1998). Metabolic activation of ME and production of 1′-hydroxy metabolite through enzymatic side-chain hydroxylation has been demonstrated as primarily responsible for hepatotox-

icity and carcinogenicity of ME (Smith et al., 2002). Earlier, we speculated that introduction of fluorine into the allylic group of ME might enhance its metabolic stability (Khrimian et al., 1994). Whether or not that was the case, the fluorine analog I showed reduced toxicity and reduced recombinagenicity in the yeast deletion assay compared to ME (Brennan et al., 1996). From the time NTP updated the list of carcinogens (NTP, 2002), we started to more vigorously pursue studies on fluoroanalog I as an alternative to ME for detection and control of oriental fruit fly. Here, we report on feeding behavior and metabolism of 1,2-dimethoxy-4-(3-fluoro-2-propenyl)benzene in *B. dorsalis* as well as its new, economical synthesis.

Methods and Materials

Insects

Laboratory-reared oriental fruit flies (strain: Punador initiated in 1988–1989 and monitored by mating and survival tests) were obtained from the USDA-ARS-PBARC rearing facility in Honolulu, HI, USA. Pupae (60 ml) were placed in a screened aluminum cage (30 × 30 × 30 cm) and allowed to emerge. Flies were given water, sugar, and hydrolyzed yeast protein and held in a room at 25–26°C, 50–70% rh, and a 12:12-hr light/dark cycle.

Chemicals

All reagents and solvents were purchased from Aldrich Chemical Co. unless otherwise specified. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Methylene chloride was distilled from calcium hydride. Coniferyl alcohol was prepared from ferulic acid (Quideau and Ralph, 1992), 2-(2-propenyl)-4,5-dimethoxyphenol (IV) was synthesized from 3,4-dimethoxyphenol (Benbow and Katoch-Rouse, 2001), and 3,4-dimethoxyphenylacetaldehyde was made from veratraldehyde (Ban and Oishi, 1958). Specific caution is necessary because osmium tetroxide is highly toxic and benzene is a carcinogen.

1,2-Dimethoxy-4-(3,3-difluoro-2-propenyl)benzene (V)

A 1-l three-neck flask equipped with a mechanical stirrer, dropping funnel, thermometer, and N_2 inlet was flashed with N_2 and loaded with hexamethylphosphorous triamide ($[(CH_3)_2N]_3P$, 70 ml, 0.387 mol). A solution dibromodifluoromethane (18 ml, 0.197 mol) in dry THF (350 ml) was added dropwise at 0°C under N_2 atmosphere. A thick white paste was formed close to the end of addition. The mixture was warmed to room temperature (RT) and stirred for another 30 min. A solution of 3,4-dimethoxyphenylacetaldehyde (17.4 g, 0.097 mol) in dry THF (80 ml) was added slowly at 25°C, whereupon the temperature was maintained at 25–30°C by cooling in a water bath. The yellowish suspension was stirred 1 hr at RT (or until TLC showed the completion of reaction), poured into the ice water (~600 ml), and extracted with hexane/ether, 1:1 (4 × 200 ml). The organic layer was washed with water (2 × 100 ml), dried (Na_2SO_4), and evaporated. The remainder was distilled in vacuum to furnish 18.03 g (87%) V of 98% purity. ^{1}H NMR: 3.27 (br. d, J = 8.0 Hz,

CH_2), 3.85 and 3.87 (both s, CH_3O), 4.37 (dtd, $J_{HF\text{-}trans}$ = 25.0, $J_{HF\text{-}cis}$ = 2.0 Hz, CH_2CH=C), 6.65–6.85 (m, arom, 3H). GC-MS (EI): 214 (M^+, 100%), 199 (12), 183 (31), 77 (19). Anal. found: C 61.54; H 5.61. $C_{11}H_{12}F_2O_2$ requires C 61.67 and H 5.66.

1,2-Dimethoxy-4-(3-fluoro-2-propenyl)benzene (I)

A solution of Red-Al® (48 ml, 70% w/w in toluene, d = 1.036, 3.4 M) was added slowly to a solution of V (17.00 g, 0.08 mol) in dry benzene (350 ml) at 0–5°C under N_2. Reaction mixture was warmed to 25°C and stirred for 20 hr or until GC indicated the completion of reduction. The mixture was poured into ice water and acidified with concentrated HCl. The organic layer was separated, and the aqueous was extracted with ether/hexane, 1:1 (2 × 100 ml). Combined organic extracts were washed with brine, dried, and distilled to give 14.50 g (92%) I (99% pure, *E*/*Z* 88:12); b.p. 55–56°C/0.05 mm Hg. ^{1}H NMR and MS spectra were identical to those described earlier (Khrimian et al., 1994).

2-(*tert*-Butyldimethylsilyloxy)-4,5-dimethoxyphenylacetaldehyde (VI)

Triethylamine (2.96 g, 29.25 mmol) followed by *tert*-butyldimethylsilyl triflate (6.19 g, 23.4 mmol) was added to a solution of IV (3.78 g, 19.50 mmol) in CH_2Cl_2 (40 ml) at 0°C. The mixture was allowed to warm to RT and was diluted after 2 hr with ether (150 ml) and washed with brine (3 × 30 ml). The organic layer was dried (Na_2SO_4), filtered, concentrated *in vacuo*, and purified by flash chromatography on SiO_2 (hexanes/ethyl acetate, 20/1 to 5/1) to yield a silyl ether of I (5.34 g, 89% yield, 99% pure). GC-MS (EI): 308 (M^+, 93%), 251 (100), 223 (76), 220 (27), 177 (20), 73 (68). A solution of OsO_4 (508 mg, 2 mmol) in acetone (40 ml) was added to a solution of the silyl ether (3.08 g, 10 mmol) in ether (200 ml). The resulting dark-brown solution was stirred for 10 min prior to addition of water (200 ml). Finely powdered $NaIO_4$ (21.39 g, 0.1 mol) was added in five portions over a period of 5 hr. The mixture was stirred for additional 3 hr, diluted with ether (100 ml), and the layers were separated. The organic phase was washed with brine, dried (Na_2SO_4), and purified by flash chromatography on SiO_2 (hexane/ethyl acetate, 10:3) to yield VI as a brown oil (2.50 g, 81%) of 99% purity. GC-MS (EI): 310 (M^+, 25%), 281 (21), 253 (100), 238 (37), 222 (74), 209 (16), 195 (30), 75 (49), 73 (75).

1-(*tert*-Butyldimethylsilyloxy)-4,5-dimethoxy-2-(3,3-difluoro-2-propenyl)benzene (VII)

CF_2Br_2 (1.52 ml, 16.65 mmol) was added to a solution of hexamethylphosphorous triamide (6.0 ml) in THF (30 ml) at 0°C. The mixture was stirred at RT for 1 hr, and a solution of aldehyde VI (2.53 g, 8.20 mmol) in THF (10 ml) was added. After stirring for 1.5 hr and workup described for the compound V, the crude mixture was chromatographed (hexane/ethyl acetate, 8:1) to furnish benzene VII (1.81 g, 64%) of 99% purity. ^{1}H NMR: 0.21 [s, 6H, $Si(CH_3)_2$], 1.00 (s, 9H), 3.21 (br. d, 2H, J = 8.0 Hz, CH_2), 3.80 and 3.82 (both s, CH_3O), 4.33 (dtd, $J_{HF\text{-}trans}$ = 25.0, $J_{HF\text{-}cis}$ = 2.0 Hz, CH_2CH=C), 6.38 (s, 1H, arom), 6.62 (s, 1H, arom). GC-MS (EI): 344 (M^+, 51%), 243 (100), 77 (18), 73 (20). Anal. found: C 59.22; H 7.88. $C_{17}H_{22}F_2O_3Si$ requires C 59.27 and H 7.61.

2-(3-Fluoro-2-propenyl)-4,5-dimethoxyphenol (II)

A solution of difluorobenzene **5** (1.758 g, 5.11 mmol) and Red-Al® (4.46 ml of 3.33 M in toluene, 14.85 mmol) in dry benzene (20 ml) was refluxed, and the reaction progress was monitored by GC. After about 48 hr, the mixture was taken into ice water, acidified with 20% HCl, and extracted with ether/hexane, 1:1. Combined organic extracts were washed with brine, dried, and concentrated. The remainder was treated at 0°C with a THF solution of tetrabutylammonium fluoride (3.0 ml, 1 M) and gradually warmed to RT to complete the removal of TBDMS protecting group. Water was added, and the products were extracted with ether, dried, and concentrated. Flash chromatography on SiO_2 with hexane/ethyl acetate, 1:1, afforded phenol II (0.960 g, 89%) with 87:13 ratio of *E* and *Z* isomers. M.p. 68–69°C. ^{1}H NMR: 3.18 (br. d, 2H, J = 7.2 Hz, CH_2-*E*), 3.37 (br. d, 2H, J = 7.2 Hz, CH_2-*Z*), 3.80 and 3.81 (both s, CH_3O), 4.70 (OH), 4.92 (dtd, $J_{HF\text{-}trans}$ = 46.0, $J_{HH\text{-}cis}$ = 4.2 Hz, CH_2CH-*Z*), 5.53 (ddt, $J_{HF\text{-}cis}$ = 18.2, $J_{HH\text{-}trans}$ = 11.0 Hz, CH_2CH-*E*), 6.41 and 6.61 (both s, arom, II-*E*), 6.43 and 6.64 (both s, arom, II-*Z*), 6.56 (br. dd, J_{HF} = 85.5, $J_{HH\text{-}trans}$ = 11.0 Hz, CF*H*-*E*). GC-MS (EI) II-*E*: 212 (M^+, 100%), 197 (21), 181 (9), 69 (27), 59 (19). *Z* isomer had almost identical mass spectrum. Anal. found: C 62.26; H 6.36. $C_{11}H_{13}FO_3$ requires C 62.26 and H 6.17.

Analytical Procedures

^{1}H NMR spectra were recorded at 300 MHz in $CDCl_3$ with TMS as an internal standard on a Bruker QE-300 spectrometer. Chemical shifts are reported in δ units. GC analyses were performed on a Shimadzu 17A gas chromatograph equipped with a flame ionization detector, an autosampler AOC-20s, and autoinjector AOC-20i. Hydrogen was used as carrier gas at 1.2 ml/min. RTX-1701 column (60 m × 0.25 mm, Restek Corporation) was used for routine analyses of synthesized and reference chemicals. HP-5 column (30 m × 0.25 mm × 0.25 μm film) was used for quantitative analyses of fly extracts in a splitless mode. Column temperature was maintained at 100°C for 2 min and then raised to 260°C at 10°C/min. Electron ionization (EI) mass spectra (70 eV) were obtained with an Agilent Technologies 5973 mass selective detector interfaced with 6890 N GC system equipped with a 30 m × 0.25 mm i.d. × 0.25 μm film HP-5MS column. Helium was used as the carrier gas at 1 ml/min. TLC analyses were conducted on Whatman AL SIL G/UV plates using hexane/ethyl acetate as a mobile phase. For visualization of spots, 20% ethanol solution of phosphomolybdic acid and/or UV light was used. Elemental analyses were conducted by Galbraith Laboratories (Knoxville, TN, USA).

Bioassay

In test no. 1, 50 laboratory-reared mature oriental fruit-fly males were evenly placed in five ~2-lb plastic containers (10/container) equipped with water inserts and screened covers. Flies in two containers were offered 10 μl ME (2 × 5 μl) on a slip of filter paper (25 × 5 mm) taped to a blank water pot. Twenty flies in two other cages were offered the fluorine analog I (10 μl/cage), and 10 males in the last cage were presented with a filter paper containing 10 μl water. The behavioral observations

(landing and feeding on the source) were recorded, and filter papers were removed after about 20 min. Water containers were replaced, and the flies were left overnight with a sugar cube on the cover. After 18 hr, the rectal glands of 10 flies each fed with ME and I as well as 10 control flies were dissected and placed in conical plastic vials with 20 μl ethanol. The glands were crushed with a wire rod, sealed with parafilm, and centrifuged at ~4000 rpm for 10 min. Six hours later (or 24 hr into the test), the remaining 10 flies fed with ME and I were dissected. Three flies fed with fluorine analog I were dead at the time of second dissection. All 20 extracts were analyzed by GC-MS by splitless injection of 1-μl solutions. The abdomens of 17 flies fed with I that were alive after 24 hr were separated from other body parts, placed in 20 μl ethanol, crashed, and analyzed by TLC using hexane/ethyl acetate, 1:1. Then, the extracts were evaporated and flash-chromatographed on a short SiO_2 column (30 × 5 mm) with hexane/ethyl acetate, 3:1. The fractions containing unmetabolized fluorine analog I were further combined, concentrated to ca. 1 ml, and quantified by GC using external standards of I. The remaining body parts (e.g., thorax, head, etc.) of 17 flies fed with I were combined, crashed, placed in ethanol (~1 ml), and analyzed by TLC. Only contents of rectal glands of ME-fed flies were analyzed by GC-MS analogous to fluorine analog I-fed flies. A standard solution of coniferyl alcohol (20 ng/μl) was used to estimate the quantities of metabolites.

Test no. 2 was conducted analogously to test no. 1 with 10 flies presented with 10 μl of ME, another 10 flies offered 10 μl of fluorine analog I, and 10 flies offered water as control. The presentation time was 40 min. After 24 hr, rectal glands were dissected, placed in 100 μl EtOH, treated as described above, and analyzed by GC with external standards of III, IV, and II. The other body parts for each fly, including abdomens, were individually placed in 20 μl EtOH, analyzed by TLC, and then separately chromatographed on SiO_2 as described in test no. 1. The fractions containing starting ME and I were analyzed by GC using external standards.

In test no. 3, 10 flies were presented with 1.0 μl (10 μl of 10% v/v hexane solution) of ME and I, and 10 flies were offered water as a control. After 24 hr, rectal gland extracts were obtained in 220 μl EtOH and analyzed by GC using standards of III, IV, and II. Abdomens were separated from other body parts, extracted with 200 μl ether, evaporated to ~50 μl, and analyzed by TLC. The abdomen extracts from FA-fed flies were further analyzed by GC. The other body parts were taken into 200 μl ether, evaporated to about 50 μl, and analyzed by TLC.

Data Analysis

Mean ratios were analyzed by using PROC GLM followed by a Tukey's test for mean separation. Significant differences were determined at the $P < 0.05$ level. Analysis was run on SAS version 8.2 (SAS Institute, 1990, Cary, NC, USA).

Results

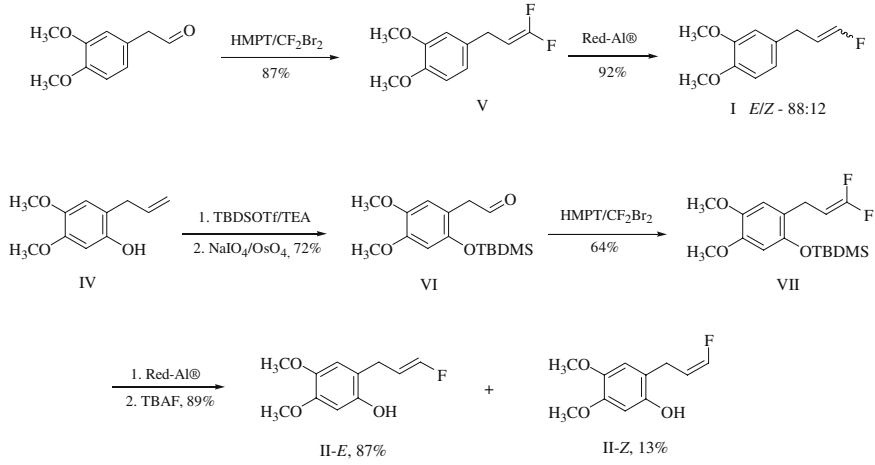
Synthesis

Earlier, we synthesized both *E* and *Z* isomers of 1,2-dimethoxy-4-(3-fluoro-2-propenyl)benzene (I) utilizing Wittig reactions of 3,4-dimethoxyphenylacetaldehyde

with stabilized ylides (Khrimian et al., 1994). Although the methods proved satisfactory for small syntheses, we envisioned difficulties in scaling up, especially in the case of I-*E* isomer, the synthesis of which required expensive reagents and multiple chromatographic purifications. Now we report on a more efficient two-step synthesis of I in a total 80% yield form 3,4-dimethoxyphenylacetaldehyde (Scheme 1). Our new synthesis utilized a straightforward Wittig difluoroolefination with a subsequent selective reduction of intermediate difluoroolefin (Hayashi et al., 1979). Notably, both intermediate and final products were purified via distillation that renders the method amenable for scale-up. We obtained about the same ratio of I-*E* and I-*Z*, 88:12, as in our previous approach and plan to continue field studies with this material, which is now being manufactured by a contractor. The same synthetic strategy was utilized to make the principal metabolite II that is formed by feeding *B. dorsalis* males with fluorobenzene I. Synthetic allylphenol IV, which happened to be a key metabolite of oxidative biotransformation of ME in *B. dorsalis*, served as a starting material in the second synthesis (Scheme 1). A TBDMS protection of phenol IV with subsequent oxidative cleavage of the double bond following Benbow and Katoch-Rouse, 2001 furnished aldehyde VI in a 72% isolated yield. Difluoroolefination of VI with HPMT/CBr_2F_2 followed by selective reduction of VII with Red-Al® gave the mixture of II-*E* and II-*Z* in 87:13 ratio. GC retention times of the geometric isomers and respective mass spectra matched those isolated from *B. dorsalis*.

Feeding Behavior and Metabolism

In test no. 1, we noticed that eight to nine *B. dorsalis* males touched the filter paper impregnated with 10 μl of I and started feeding on it. In case of ME, the number of flies that contacted the source was five to six, and no flies landed on filter paper treated with water. After about 20 min, the flies left the source in both treatments, and feeding was discontinued. By the time of dissection of first 10 individuals (18 hr), all flies in both treatments and in control were healthy, but



Scheme 1 Syntheses of the fluorine analog I and the principal metabolite II

after another 6 hr, three flies fed with fluorine analog I died. GC-MS analysis of the rectal glands of all 20 *B. dorsalis* males fed with I (including dead flies) showed three main peaks, one of which was easily identified as (*E*)-coniferyl alcohol (III) matching with the standard by retention time (13.46 min) and mass spectrum. The other two (RT 12.70 and 12.97 min) had almost identical mass spectra with a strong ion at 212 *m*/*z* that could have been attributed to a molecular ion of *E* and *Z* fluoroolefinic phenols because of similarities with the mass spectrum of IV (Nishida

Table 1 Amounts of metabolites and unmetabolized methyl eugenol and fluorine analog I in individual oriental fruit-fly species from test no. 2[a]

Methyl eugenol (ME)				
Insect no.	Metabolites[b] (μg)		IV/III (mol/mol)	Unmetabolized, ME[c] (μg)
	IV	III		
1	9.6	9.3	1.0	235.0
2	9.7	10.2	0.9	99.0
3	3.0	5.6	0.5	86.8
4	10.5	13.8	0.7	25.5
5	26.3	24.8	1.0	253
6	1.3	1.1	1.1	52.6
7	19.5	26.7	0.7	801.5
8	9.6	12.0	0.7	133.2
9	15.9	19.8	0.7	268.0
10	9.9	11.7	0.8	128.7
Total	250.3		2083	
Mean ± SEM	11.5 ± 2.3	13.5 ± 2.5	0.8 ± 0.1 b[d]	208 ± 71
Fluorine analog I				
Insect no.	Metabolites[b] (μg)		II/III (mol/mol)	Unmetabolized, I[c] (μg)
	II (*E* + *Z*)	III		
1	5.5	1.3	3.6	482
2	5.3	0.6	7.6	1.4
3	17.6	2.1	7.1	1014
4	5.3	0.8	5.7	n/d
5	9.4	1.3	6.2	681
6	6.9	1.3	4.5	28.3
7	3.7	0.4	7.9	30.5
8	10.7	1.6	5.7	863
9	18.6	2.4	6.6	1082
10	4.1	0.6	5.8	587
Total	99.5		4769.2	
Mean ± SEM	8.7 ± 1.7	1.2 ± 0.2	6.1 ± 0.4 a[d]	529 ± 142

[a] Ten flies were presented with 10 μl ME and analog I.

[b] Found in rectal glands.

[c] Total in other body parts.

[d] Means followed by different letters are significantly different at the 0.05 level, PROC GLM, Tukey's test (SAS Institute, Version 8.2).

et al., 1988a,b). Having synthesized the targeted products, we identified these peaks as II-*E* and II-*Z*. In addition to three main peaks, there were some minor products in these rectal gland extracts but no starting I. However, unmetabolized I was found in fairly large quantities in the abdomen of the flies. In the total 17 males dissected, we found 2948 μg starting fluorinated benzene I (or in average 173 μg per fly) indicating an intense feeding. Finally, there were no phenylpropanoids found in other body parts of the flies fed with fluorine analog I. All 20 flies exposed to ME accumulated phenol IV and coniferyl alcohol III in their rectal glands as expected (Nishida et al., 1988a,b), in some cases in fairly large quantities (15–20 μg). There were no phenylpropanoids found in control flies.

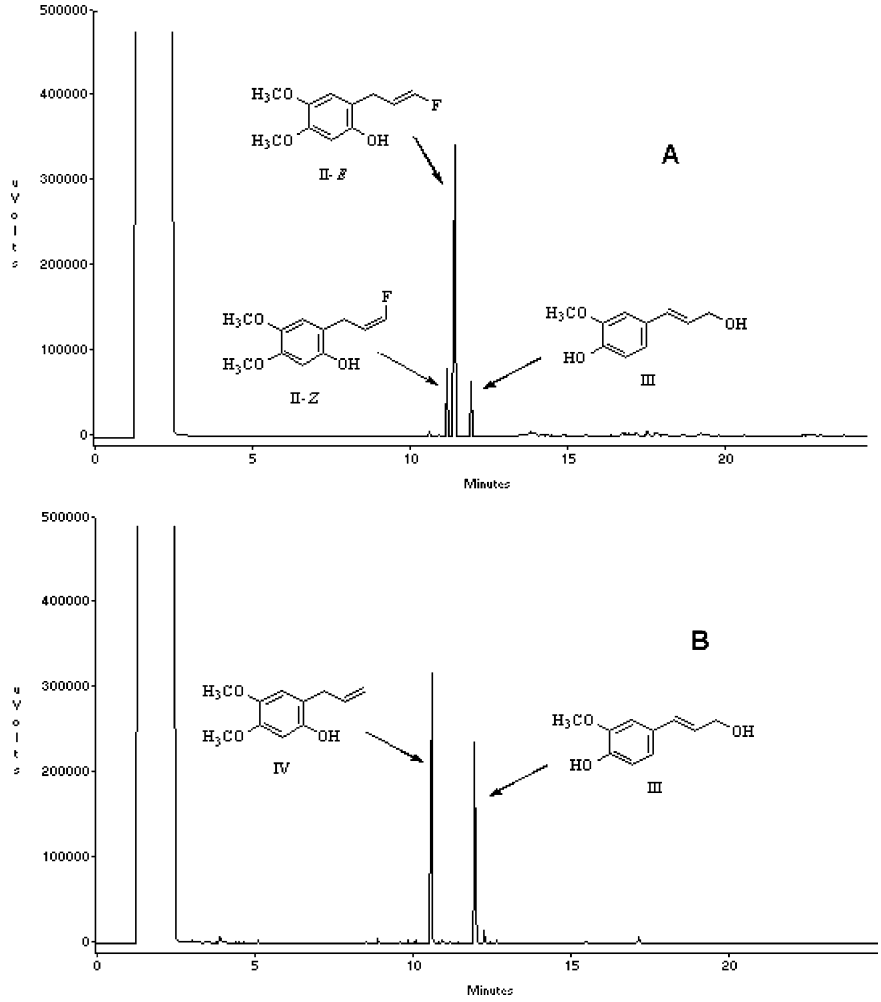


Fig. 1 Gas chromatograms (HP-5, 30 m × 0.25 mm × 0.25 μm, FID) of gland extracts of individual oriental fruit fly males fed with: (A) fluorine analog I (Table 1, species no. 3, II + III = 19.7 μg; II/III = 7.1, mol/mol) and (B) methyl eugenol (Table 1, species no. 1, IV + III = 18.9 μg; IV/III = 1, mol/mol)

In test no. 2, we repeated the experiment with 10 μl of each ME and benzene I and ran the feeding for 40 min. In both treatments, five to six flies initially landed on filter papers; then after 15–20 min, all flew away. In the last 15 min, some flies returned to feeding sources, but we had no knowledge whether all flies touched the filter papers. Dissection of rectal glands was conducted after 24 hr. Quantitative analyses of metabolites as well as unmetabolized ME and fluorine analog I in individual flies are presented in Table 1. With the exception of two species (nos. 2 and 4) that apparently did not touch the source, the rest of fluorine analog-fed *B. dorsalis* males contained large amounts of unmetabolized material in their abdomens, ranging from 28 μg up to ~1 mg. Remarkably, a total of 10 flies consumed the fluorinated benzene I (4769.2 ± 99.5 μg) in more than twice the amount of ME (2083.3 ± 250.3 μg), but produced about half the amount of metabolites (99.5 vs. 250.3). The other striking difference was the ratio of metabolites derived from ME and fluorinated benzene I. The average molar ratio of phenol IV and coniferyl alcohol III (Table 1) was 0.8 (±0.1 SEM), whereas the average ratio II/III was 6.1 (±0.4 SEM). Gas chromatograms of the rectal gland extracts of two males (one fed with ME and another with benzene I) that produced about the same total amounts of metabolites are presented in Figure 1.

In test no. 3 with 1.0 μl of ME and fluorine analog I, with the exception of one fly fed with analog I, there were no unmetabolized starting materials after 24 hr. The amounts and ratios of metabolites are presented in Table 2. Analogous to the previous test, a total of 10 flies consumed nearly twice as much the fluorine analog I as ME (9.10 vs. 4.58 μg). But in this low-dose consumption, both test compounds

Table 2 Amounts of metabolites in individual oriental fruit-fly males fed with methyl eugenol and fluorine analog I from test no. 3[a]

Insect no.	Methyl eugenol (ME) metabolites[b]			Fluorine analog I metabolites[b]		
	IV (ng)	III (ng)	IV/III (mol/mol)	II (*E* + *Z*) (ng)	III (ng)	II/III (mol/mol)
1	126	20	5.8	326	36	7.7
2	213	55	3.6	780	60	11.0
3	125	37	3.1	600	39	13.0
4	371	313	1.1	419	39	9.1
5	194	33	5.5	1135[c]	191	5.0
6	248	174	1.3	486	43	9.6
7	549	628	0.8	418	50	7.1
8	64	20	3.0	613	45	11.6
9	734	482	1.4	n/d	n/d	
10	117	77	1.4	1851	148	10.
Total	4580			9103		
Mean ± SEM	274 ± 68	183 ± 69	2.7 ± 0.6 b[d]	736 ± 161	72 ± 18	9.4 ± 0.8 a[d]

[a] Ten flies were offered 1.0 μl of ME and I.

[b] Found in rectal glands. With the exception of one fly fed with I (line no. 5), no unmetabolized ME or analog I was found.

[c] 1893 ng of I was found in the abdomen.

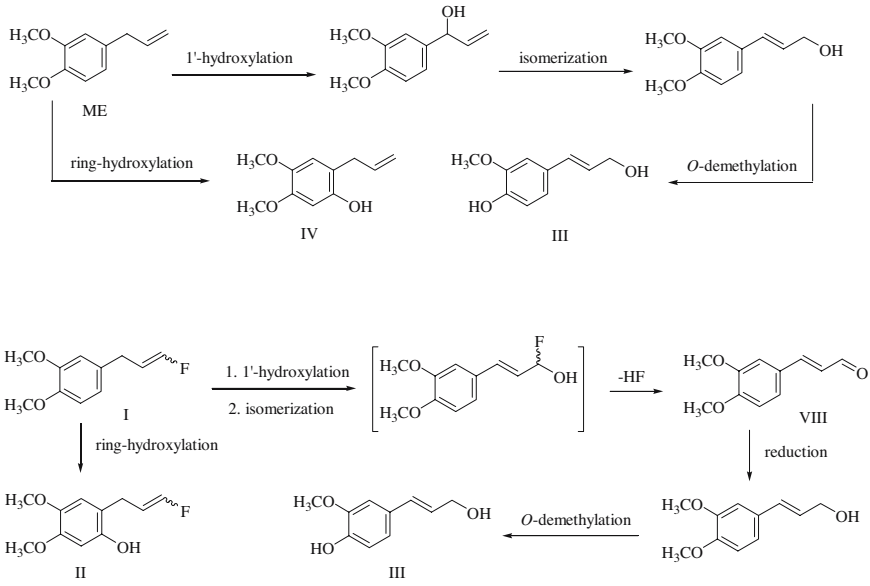
[d] Means followed by different letters are significantly different at the 0.05 level, PROC GLM, Tukey's test (SAS Institute, Version 8.2).

were almost completely metabolized. Again, the mean ratio IV/III was significantly lower than the mean ratio II/III.

Discussion

Formation of coniferyl alcohol III as one of two main metabolites produced by *B. dorsalis* males after feeding on fluorine analog I indicated the occurrence of a side-chain enzymatic hydroxylation, analogously to a biotransformation of ME in the oriental fruit fly. Although mechanistic aspects of ME metabolism in *B. dorsalis* have not been articulated, there is ample information in literature on metabolism of ME in rodents (Solheim and Scheline, 1976; Smith et al., 2002) and even humans (Smith et al., 2002 and references therein). In rats, dimethoxyphenol IV was reportedly one of the major metabolites, but coniferyl alcohol III had not been found (Solheim and Scheline, 1976). In a recent review, Smith et al. (2002) outlined three major metabolic pathways of ME and related phenylpropanoids in mammals: *O*-demethylation to phenols, epoxidation of the double bond followed by hydration, and 1′-hydroxylation to form a benzylic alcohol, which often undergoes allylic rearrangement to a more stable primary alcohol. There was no mention of coniferyl alcohol even as a by-product. Surprisingly, ring hydroxylation leading to phenol IV had been overlooked. At any rate, it appears that coniferyl alcohol is a unique metabolite of ME in *B. dorsalis* and related complex sibling species (Wee et al., 2002), destined to perform a significant pheromonal and allomonal role in fruit-fly biology and ecology (Nishida et al., 1988a,b; Tan and Nishida, 1998, 1996; Wee and Tan, 2001). Mechanistically, the formation of III from ME in *B. dorsalis* could be rationalized as a combination of two major pathways: 1′-hydroxylation with further allylic isomerization and *O*-demethylation in whichever order (Scheme 2). In fact, one product in the 1′-hydroxylation pathway, *cis*-3,4-dimethoxycinnamyl alcohol, was isolated by Nishida et al. (1988b). From this standpoint, metabolism of fluorinated analog I in *B. dorsalis* would lead to a cinnamic aldehyde intermediate VIII (hypothetically through elimination of HF from a 1-fluorocinnamic alcohol) followed by a reduction and further demethylation to coniferyl alcohol III (Scheme 2). The proposed scheme explains the loss of fluorine during metabolic transformation of I to III. However, the aldehyde VIII or its *O*-demethylated analog is yet to be found in the *B. dorsalis* rectal gland extracts. An alternative route includes an enzymatic defluorination of I to form ME, which then follows 1′-hydroxylation–isomerization pathway described above leading to III. Further research will focus on finding evidence supporting either of these hypotheses.

Whether or not the death of three flies in test no. 1 after feeding on fluorine analog I could be associated with a partial metabolic loss of fluorine (in the form of HF or any other) is yet to be determined. In test no. 2 conducted with the same load of I, but even longer feeding, all flies seemed healthy. It seems also possible that feeding on large quantities of I and the inability to efficiently metabolize sicken *B. dorsalis* males, as the consumption of large amounts of even natural product ME reportedly caused the death in the oriental fruit fly (Steiner, 1952). More dose-response studies should be conducted to see whether the fitness of *B. dorsalis* males could be compromised by the intake of fluorine analog I.



Scheme 2 Suggested metabolic transformations of methyl eugenol and fluorine analog I in *B. dorsalis*

Clearly, *B. dorsalis* males perceive the analog I as strong as ME. In two experiments (test nos. 2 and 3), *B. dorsalis* males consumed even total larger quantities of I than the parent molecule. Although not all flies directly contacted the filter papers, they still consumed appreciable amounts of material through a vapor phase. Under our experimental setting, individual *B. dorsalis* males consumed up to 1 mg of I. The highest number for ME intake in *B. dorsalis* was reportedly 2.2 μl, and the average quantity was 700 μg (Wee et al., 2002). The fact that *B. dorsalis* males voraciously feed on fluorine analog I might have a practical implication because eradication programs, by and large, rely on reducing the populations of wild oriental fruit fly males by exposure of ME mixed with toxicants.

Two important observations seem interconnected. At high feed (test no. 2), 10 oriental fruit fly males metabolized after 24 hr only 2.0% of the fluorine analog I they consumed, whereas flies fed with ME metabolized 10.7% of their intake, indicating that incorporation of the fluorine atom overall impeded the metabolism. As a result, total amount of metabolites from ME was higher than from analog I. At low feed (test no. 3), the flies were still able to efficiently metabolize almost the entire intakes of the analog I (and not surprisingly, ME), which resulted in the reversal of metabolite quantities. However, in both test nos. 2 and 3 run at different doses, the fluorination unmistakably redirected the metabolism in favor of ring-hydroxylation pathway. Introduction of fluorine (as an isosteric replacement for hydrogen) in the biologically active substances including insect semiochemicals has long been pursued for altering volatility and lipophilicity, as probes for studying the insect communication mechanism, enhancing metabolic stability, and as enzyme inhibitors (for a recent review, see Pesenti and Viani, 2004). Whatever the mecha-

nism, the introduction of fluorine at the terminal atom of the double bond of ME appears to overall retard the oxidative biotransformation of this important plant phenylpropanoid in *B. dorsalis* and specifically influenced side-chain hydroxylation. It is intriguing whether the metabolism of fluorine analog I in mammals will follow the same path.

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